bio-protocol Preprint

Not Peer Reviewed
Ask a question online

Isolation of DP cells from C57BL/6 mice

SH Shiqi Hu KC Ke Cheng

Updated date: Jan 9, 2023

An abbreviated version of this protocol was published in Science Advances in Jul 2020

Dermal exosomes containing miR-218-5p promote hair regeneration by regulating β-catenin signaling

DOI: 10.1126/sciadv.aba1685

Detailed protocol

Experimental Preparation

1. Spread out a bench pad in a cell culture hood. Place 150mm cell culture plates, PBS (on ice) and dermal papilla (DP) medium in the hood.
2. Place autoclaved surgical instruments (blades, scissors and tweezers) in a 50mL conical with 25mL 70% Ethanol.
3. UV sterilize for 30 min.

Isolation of hair follicles from mouse vibrissa pads

1. Euthanize mice with a CO_2 overdose in a euthanasia chamber.
2. Shave the vibrissa hair with electric clippers.
3. Disinfect the whole mouse head with 70% ethanol.
4. Cut off the mouse vibrissa pads and place them on a surgery pad in the hood.
5. Place the vibrissa pads in cell culture plates and rinse it in PBS (on ice) three times.
6. Turn the skin side upside down, pick up the follicle bulbs from the dermis layer and cut them off. Expect to collect ~20 follicles per mouse.
7. Hair follicles are incubated with 0.25% dispase (STEMCELL Technologies) for 20 min in the incubator. Then, hair follicles were rinsed in PBS three times.
8. A small cut was made on the follicle bulb (This step can be performed under a stereo microscopy). The hair follicle bulbs were then transferred into a rat-tail collagen I (Sigma-Aldrich)–coated 12-well plates (one follicle bulb per well). 0.5 mL medium per well was added. We used Eagle's minimal essential medium (MEM; Gibco), 10% fetal bovine serum (FBS; Corning), 1% penicillin-streptomycin, and bFGF (10 ng/ml; Fisher Scientific) as DP medium.
9. Leave the plates in the incubator for 3~7 days. Obverse the plates gently since Day 3. Add another 0.5 mL fresh medium once DP cells grow out of the follicles.
10. Passage the cells in each well into a rat-tail collagen I coated T25 cell culture plate. DP cells can reach confluence in one week.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Hu, S. and Cheng, K. (2023). Isolation of DP cells from C57BL/6 mice. Bio-protocol Preprint. bio-protocol.org/prep2110.
2. Hu, S., Li, Z., Lutz, H., Huang, K., Su, T., Cores, J., Dinh, P. C. and Cheng, K.(2020). Dermal exosomes containing miR-218-5p promote hair regeneration by regulating β-catenin signaling . Science Advances 6(30). DOI: 10.1126/sciadv.aba1685

Copyright: Content may be subjected to copyright.